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ARTICLE

MORPHOLOGICAL AND HISTOLOGICAL EVIDENCE FOR THE OLDEST KNOWN SOFTSHELL TURTLES FROM JAPAN

YASUHISA NAKAJIMA,*[,1,2] IGOR G. DANILOV,[3,4] REN HIRAYAMA,[5] TEPPEI SONODA,[6] and TORSTEN M. SCHEYER[7]

[1]Atmosphere and Ocean Research Institute, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa-shi, Chiba 277-8564, Japan, ynakajima@aori.u-tokyo.ac.jp;

[2]Steinmann Institute for Geology, Mineralogy and Paleontology, University of Bonn, 53115 Bonn, Germany;

[3]Department of Herpetology, Zoological Institute, Russian Academy of Sciences, Universitetskaya Emb. 1, St. Petersburg 199034, Russia, igordanilov72@gmail.com;

[4]Department of Vertebrate Zoology, Saint Petersburg State University, Universitetskaya Emb. 7/9, St. Petersburg, 199034, Russia;

[5]School of International Liberal Studies, Waseda University, 1-6-1 Nishiwaseda, Shinjuku-ku, Tokyo 169-8650, Japan, renhirayama@gmail.com;

[6]Fukui Prefectural Dinosaur Museum, 51-11 Terao, Muroko, Katsuyama, Fukui 911-8601, Japan, t-sonoda@dinosaur.pref.fukui.jp;

[7]Universität Zürich, Paläontologisches Institut und Museum, Karl Schmid-Strasse 4, CH-8006 Zürich, Switzerland, tscheyer@pim.uzh.ch

ABSTRACT—Herein we report morphologically and histologically diagnostic trionychid specimens from the Barremian–Aptian (129.4–113.0 Ma) of Japan. One specimen (FPDM-V9487) is an associated skeleton that consists of a scapula, a humerus, an ischium, and hypoplastra. The limb and girdle bones are similar in morphology to those of modern trionychids. The hypoplastron also resembles that of modern trionychids; however, it is distinctive in that it completely lacks callosities. The other three new specimens are fragmentary costals that show a reduction in the shell bones and an absence of scute sulci. Two of the newly reported costals and FPDM-V0127 were examined histologically. Bone fiber bundles organized in a plywood-like structure, which is unique to trionychids, were identified. Fossil occurrence data indicate that morphologically and histologically typical trionychids already inhabited the coastal region of Asia (e.g., western Japan) as early as the Aptian. In contrast, the Hauterivian–Aptian stem trionychid *Kappachelys okurai* from Japan does not show a plywood-like shell microstructure, suggesting that *K. okurai* could be the earliest-branching taxon of known stem trionychids or a carettochelyid. Our paleobiogeographic compilation suggests that the spread of wetlands in the Northern Hemisphere and the high global temperature during the middle Cretaceous (Aptian–Turonian, 125.0–89.8 Ma) might have contributed to the dispersal of trionychids from Asia, allowing for the establishment of this modern freshwater reptilian fauna.

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INTRODUCTION

Trionychids (softshell turtles) are highly aquatic, non-marine cryptodiran (hidden-necked) turtles that currently live in ponds, lakes, or slow-moving water in Asia, Africa, North America, and the Indo-Australian archipelago. This group is one of the most successful turtle families, with an almost worldwide distribution in the fossil record including all major continents except Antarctica (Gaffney and Bartholomai, 1979; Meylan et al., 1990; Head et al., 2006). The body plan of trionychids is distinct from that of other non-marine turtles; scales and keratinous scutes are totally absent, the body is extremely flattened, and carapacial/plastral elements are reduced to form a partly flexible shell. All these characteristics have been identified as adaptations to aquatic environments (Stone et al., 1992; Renous et al., 2007).

Trionychidae (in this study, we include crown and stem trionychids when using this term, equivalent to Pantrionychidae of Joyce et al., 2004) are commonly found from Lower and Upper Cretaceous deposits in east and central Asia (Hirayama et al., 2000; Hutchison, 2000; Danilov and Vitek, 2012; Danilov et al., 2014). In North America and Europe, trionychids did not become common until the Late Cretaceous (Fiorillo, 1999; Brinkman, 2003; Scheyer et al., 2012, see also Discussion). During the late Cenozoic, trionychids were truly cosmopolitan, having been found in both Americas, Europe, Australia, and Africa, and they only became extinct recently in South America and Australia (Gaffney and Bartholomai 1990; Meylan et al., 1990; Head et al., 2006). Most fossil occurrences of Trionychidae are fragmentary shell elements identified on the basis of their surface sculpture and lack of scute sulci, but caution is required because surface sculptures on shell bones are also present in other pantrionychian turtles (carettochelyids, nanhsiungchelyids, and adocids) (Joyce et al., 2013), and a partial or complete loss of carapacial scutes is found in both Trionychidae and Carettochelyidae (Joyce, 2007; Hirayama et al., 2012).

A single fragmentary costal bone (FPDM-V0127) was collected from a vertebrate fossil locality, the 'Kitadani Dinosaur Quarry,' in the Kitadani Formation (Tetori Group) exposed along the Sugiyamagawa River, Katsuyama, Fukui Prefecture,

*Corresponding author.

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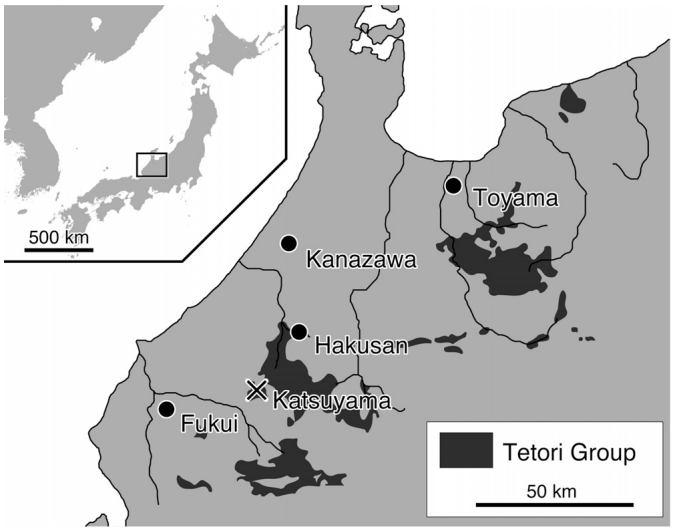

FIGURE 1. The distribution of the Lower Cretaceous Tetori Group in southwestern Japan, redrawn following Sano (2015).

Japan (Fig. 1). This specimen was identified as a trionychid on the basis of the pitted surface sculpture on its shell plate, except at the distal margin, and the lack of scute sulci (Hirayama, 2002). The Kitadani Formation has been dated to the Barremian–Aptian (129.4–113.0 Ma) on the basis of the charophyte flora and molluscan assemblage from exposures of this formation close to the Kitadani Dinosaur Quarry (Isaji, 1993; Kubota, 2005; Sano et al., 2008; Cohen et al., 2013; Sano, 2015); however, no radiometric age data are known from this formation. Recently, a nearly complete trionychian specimen from the Jiufotang Formation (Aptian) of China, representing a new genus and species, *Perochelys lamadongensis*, was reported (Li et al., 2015a). Despite the geological age of the locality, it is difficult to determine whether this species is a stem or a crown trionychid because of the high levels of homoplasy among trionychids (Li et al., 2015a). '*Trionyx' jixiensis* from the Chengzihe Formation (Aptian–Albian) of northeastern China also possessed a carapace similar to that of crown trionychids (Li et al., 2015b). '*Trionyx' kyrgyzensis* from the lower–middle Albian of the Kylodzhun locality, Kyrgyzstan (Nessov, 1995), is the oldest known trionychid outside East Asia. Therefore, specimen FPDM-V0127 is tentatively thought to be one of the oldest records of Trionychidae. At this stage, however, only a few characteristics of a single fragment support the validity of the record. Despite the importance of and necessity for reexamination, most turtle specimens from the Kitadani Formation are yet to be described.

Another candidate for the oldest trionychid is *Kappachelys okurai* from the lower part of the Akaiwa Formation (Tetori Group) in Hakusan, Ishikawa Prefecture, Japan (Hirayama et al., 2012; Fig. 1). The Akaiwa Formation is thought to underlie the Myodani Formation, which is often correlated lithostratigraphically with the Kitadani Formation (e.g., Maeda, 1958), and the zircon U–Pb age estimate for the lower part of the underlying Kuwajima Formation is 130.7 ± 0.8 Ma, which corresponds to the Hauterivian (Kusuhashi, 2008; Cohen et al., 2013). Therefore, the depositional age of the Akaiwa Formation is likely to be within the Hauterivian–Barremian interval if the Myodani Formation is contemporaneous with the Kitadani Formation and the Kitadani Formation includes the Barremian. However, there is controversy concerning the upper limit of the Akaiwa Formation. A recent zircon U–Pb age estimate of a tuff bed intercalated in the Akaiwa Formation yielded 121.1 ± 1.1 Ma, which corresponds to the Aptian (Sakai et al., 2015). The correlation between the Kitadani and Myodani Formations is also controversial. Some authors correlate the Kitadani Formation in Katsuyama with the Kuwajima Formation in Shiramine and not with the younger Myodani Formation (Matsukawa et al., 2006, 2009; Sano et al., 2008). Here, we consider the age of the specimens from the Kitadani Formation and *K. okurai* from the Akaiwa Formation to be Barremian–Aptian and Hauterivian–Aptian, respectively. *Kappachelys okurai* is known from two fragmentary carapacial elements. This turtle is thought to be a stem trionychid because its known carapace elements have deep surface ornamentation and lack scute sulci, as in trionychids; however, the species still retains robust peripheral bones, which are present in non-trionychid turtles but have been lost or reduced in trionychids (Hirayama et al., 2012; see also the discussion in Delfino et al., 2010).

Recent studies have reported that the histology of trionychid shell bones shows a layer with a characteristic plywood-like arrangement of fiber bundles (Scheyer et al., 2007, 2012; Vlachos et al., 2015; de Buffrénil et al., 2016). Because this shell bone microstructure is present in Trionychidae and absent in Carettochelyidae (the sister taxon of Trionychidae; we use this term to include the only extant member, *Carettochelys insculpta*, and all the stem taxa, equivalent to Pancarettochelys in Joyce et al., 2004) and all other turtles, this characteristic is likely to be a highly reliable synapomorphy of Trionychidae (Scheyer et al., 2007; Joyce and Lyson, 2010).

MATERIALS AND METHODS

To verify the occurrence of trionychids from the Hauterivian–Aptian of Japan, we describe trionychian fossil material from the Kitadani Formation that is currently stored in the Fukui Prefectural Dinosaur Museum (FPDM). In addition, we perform histological comparisons of the Hauterivian–Aptian trionychian specimens from the Akaiwa and Kitadani formations of Japan with other known Cretaceous–extant trionychians to determine whether the oldest known trionychid candidates also possess the synapomorphic microstructure of Trionychidae.

In this study, we compiled a data set of non-marine turtles from the Hauterivian–Turonian using previously published studies and the Paleobiology Database (https://paleobiodb.org). Fossil records for which the locality information was not available were not included in this compilation. The production of paleomaps and the reconstruction of paleocoordinates of the fossil localities were conducted based on the global plate tectonic model proposed by Hay et al. (1999) using the Advanced Plate Tectonic Reconstruction Service provided by the Ocean Drilling Stratigraphic Network (http://www.odsn.de/odsn/services/paleomap/adv_map.html).

For histological analysis, shell bone materials were collected from Cretaceous trionychid fossils, including *Kappachelys okurai*, specimens held in the FPDM, and Aptian–Maastrichtian trionychids from Asia, as well as from extant trionychids and extant/fossil carettochelyids for comparative purposes (Appendix 1). Fossil turtle shell materials were preferentially sampled from costal bones because they were available for all the examined taxa. Additional materials, including carapacial, plastral, and indeterminate shell elements, were also sampled if available. The specimens were sectioned using a diamond saw blade so that the sectional plane was perpendicular to the shell surface. Costal bones were cut in a plane perpendicular to the longitudinal axis of the corresponding rib (Fig. 4A). Other carapacial bones, i.e., the nuchal, neural, and peripheral (including the posterior ossicles of *Lissemys*) bones, were sectioned in the midsagittal, coronal, and radial planes, respectively. Plastral bones were sectioned in the parasagittal plane if determinable. For indeterminate bones, the orientation of the vertical sectional

plane was not consciously determined. Bone segments were embedded in epoxy resin under vacuum and processed into petrographic thin sections using standard procedures (e.g., Scheyer et al., 2007). Thin sections were analyzed under cross-polarized light using a Leica DMLP microscope at the Steinmann Institute for Geology, Mineralogy, and Paleontology, University of Bonn, Bonn, Germany. Photomicrographs were taken using a Leica DFC 420 camera. We also calculated compactness of the most complete section of a trionychid costal from the Kitadani Formation (FPDM-V0127), comparative costal specimens from three other trionychids, and *C. insculpta*. In the compactness analysis, we used manually binarized micrographs and Bone Profiler version 4.5.8 (Girondot and Laurin, 2003).

Institutional Abbreviations—FM(NH), The Field Museum, Chicago, Illinois, U.S.A.; **FPDM**, Fukui Prefectural Dinosaur Museum, Katsuyama City, Fukui Prefecture, Japan; **HLMD**, Hessisches Landesmuseum Darmstadt, Darmstadt, Germany; **MAGNT**, Museum and Art Gallery of the Northern Territory, Darwin, Australia; **NSMT**, National Museum for Nature and Science, Tokyo, Japan; **NUM**, Nagoya University Museum, Nagoya, Japan; **SBEI**, Shiramine Board of Education, Hakusan City, Ishikawa, Japan; **SMNS**, Staatliches Museum für Naturkunde Stuttgart, Stuttgart, Germany; **UB**, University of Bonn, Bonn, Germany; **UCMP**, Museum of Paleontology, University of California at Berkeley, Berkeley, California, U.S.A.; **WU**, Waseda University, Tokyo, Japan; **YPM**, Peabody Museum of Natural History at Yale University, New Haven, Connecticut, U.S.A.; **ZIN**, Zoological Institute, Russian Academy of Sciences, Saint Petersburg, Russia.

RESULTS

Morphology of the New Specimens

Associated plastral elements (right and left hypoplastra), a left humerus, a left scapula, and a right pubis (FPDM-V9487) were collected from the Katsuyama Dinosaur Quarry, where the only known trionychid specimen from the Kitadani Formation (FPDM-V0127, costal fragment) was found (Fig. 2). Three isolated carapacial elements (FPDM-V2761, costal; FPDM-V6370, right seventh costal; and FPDM-V1836, left eighth costal) were also collected from the same quarry. All the shell specimens lack scute sulci, suggesting a complete, or at least partial, loss of the carapacial/plastral scutes (Fig. 3).

The hypoplastron of FPDM-V9487 is markedly reduced and narrow anteroposteriorly, especially in the middle part, between the medial and lateral margins, and possesses two robust striated lateral processes and three or more fragile medial processes (Fig. 2A, B). The external surface of the hypoplastron lacks prominent ornamentation (Fig. 2A), similar to *P. lamadongensis* (Li et al., 2015a). The outline of the hypoplastron closely resembles that of '*T.' kyrgyzensis* and *P. lamadongensis*; however, it is distinguishable from them and all other Cretaceous trionychids in that this element does not bear any callosities (Nessov, 1995; Vitek and Danilov, 2014; Li et al., 2015a). The scapular acromion process lacks a medial end, and the fracture cross-section is dorsoventrally compressed; the preserved part of the acromion process is robust and curved to form a convex ventral aspect; and the main body of the scapula is longer than the preserved part of the acromion process and slightly curved to form a concave lateral aspect (Fig. 2C). The humerus lacks its distal end so that the ectepicondylar foramen is not visible; the humeral head is suboval and posterodistally long, and on both sides the large medial and lateral processes (greater and lesser trochanters in Meylan, 1987, respectively) are present, with the lateral process being plate-like and lying in an anteroposterior plane that extends through the main axis of the humerus as in modern trionychids (Meylan, 1987) and *Gobiapalone orlovi* from the Late Cretaceous (Danilov et al., 2014). The shaft of the humerus shows a gentle sigmoidal curve (Fig. 2D, E). The ischium possesses a long pointed lateral process (the metischial process in Meylan, 1987), and the lateral process is as distinct as that of *P. lamadongensis* (Li et al., 2015a) and the modern trionychid species *Chitra indica* (Meylan, 1987) or even more pronounced. The ischial extension into the thyroid fenestra is minimal; therefore, the fenestra is not likely to be divided by an ischiopubic contact (Fig. 2F).

The costals (FPDM-V2761, V6370, and V1836) are nearly flat and sculptured with dense pits on the external surface, except in the rough-surfaced distal marginal area, and the distal edge of the costal plate is rounded and lacks a suture to the peripheral bones (Fig. 3A, D, E). The lack of a costal-peripheral suture is a

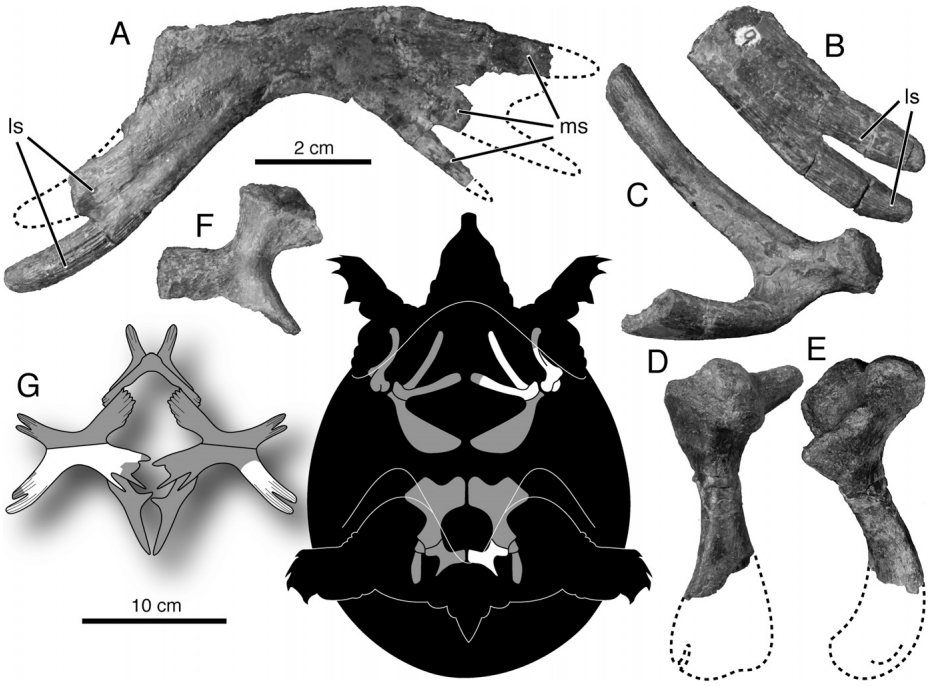

FIGURE 2. Trionychid specimens (FPDM-V9487) from the Barremian–Aptian Kitadani Formation, Tetori Group, of Japan. **A**, an almost complete right hypoplastron, external view; **B**, the lateral end of the left hypoplastron, external view; **C**, a nearly complete scapula, anterior view; **D**, **E**, the left humerus lacking distal end, dorsal (**D**) and lateral (**E**) views; **F**, the right ischium, dorsal view; **G**, a partial skeletal reconstruction of FPDM-V9487 from the ventral view. The missing parts were reconstructed based on *Perochelys lamadongensis* from the Aptian of China and '*Trionyx*' *kyrgyzensis* from the Albian of Kyrgyzstan. The body outline is based on a modern trionychid *Apalone spinifera*. **Abbreviations**: **ls**, lateral spines; **ms**, medial spines.

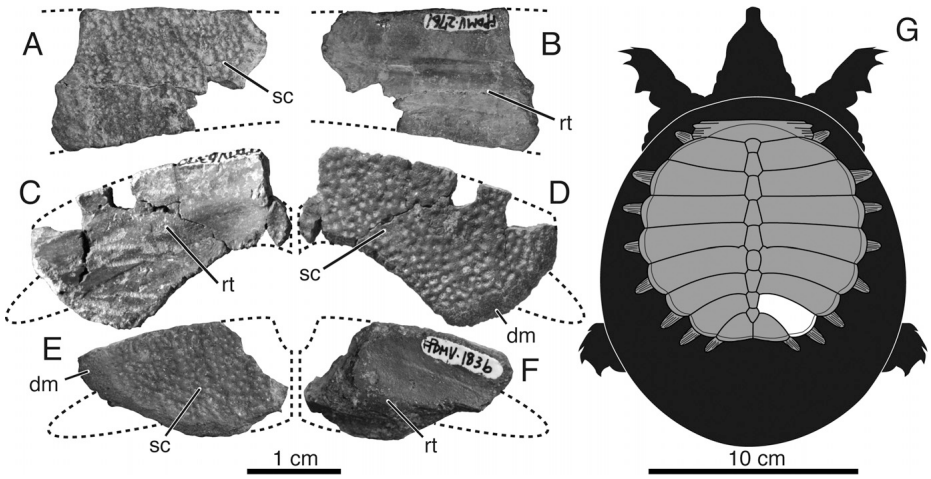

FIGURE 3. Costal specimens of trionychids from the Kitadani Formation. **A**, **B**, FPDM-V2371 (indeterminate costal), external (**A**) and internal (**B**) views; **C**, **D**, FPDM-V6370 (right seventh), internal (**C**) and external (**D**) views; **E**, **F**, FPDM-V1836 (left eighth), external (**E**) and internal (**F**) views; **G**, a carapace skeletal reconstruction with the body outline of FPDM-V6370 in the dorsal view. The missing parts were reconstructed based on '*Trionyx*' *kyrgyzensis* from the Albian of Kyrgyzstan. The body outline is based on *Apalone spinifera*. **Abbreviations**: **dm**, distal marginal area; **sc**, surface sculpture; **rt**, rib thickening.

synapomorphy shared by Trionychia (Trionychidae plus Carettochelyidae) (Meylan, 1987; Joyce, 2007). The internal surface of the costals is smooth and shows a low and wide thickening that extends proximodistally and continues to the distal end of the rib (Fig. 3B, C, F). This 'rib thickening' is characteristic of non-*Kappachelys* trionychids and is absent in Carettochelyidae, which have flat internal costal surfaces (Hirayama et al., 2012).

Histological Analysis

In all the turtles histologically examined in this study, the shell bones show a general diploë structure in which an interior layer with many cavities is sandwiched between the compact external and internal cortices (Fig. 4B, E, G). The interior layer is intensely remodeled with secondary osteons or secondary endosteal bones, with a nearly complete replacement of the primary bone tissue (Fig. 4B, G). In the interior layer, cavities are larger than in the external or internal compact layers; however, the compactness of interior tissue varies to some degree. The interior layer commonly consists of thick trabeculae and narrow intertrabecular spaces, and is not very cancellous (Fig. 4B, E, G). The trionychian shell bone internal cortex consists of parallel-fibered bone tissue that partially transitions into lamellar bone, which often exhibits distinct layered structures and is vascularized with simple canals (Fig. 4B, G). The degree of vascularity in the internal cortex is generally high in trionychids and moderate to low in carettochelyids (Fig. 4B, G). In trionychid costals, the internal cortex thickness is maximal where the rib thickening occurs (Fig. 4B).

Trionychian shell bone external cortices are often vascularized, with simple vascular canals and/or primary osteons (Fig. 4D, F–I). In most Aptian–extant trionychid shell bones, the outer zone of the external cortex consists of periosteal parallel-fibered bone tissue extending subparallel to the wavy external bone surface and is frequently associated with the vertical insertion of Sharpey's fibers (Fig. 4B, E–H). This zone is, however, only thinly present or absent in *Lissemys punctata, Plastomenus* sp., and some specimens of '*Trionyx' kyrgyzensis* (Fig. 4F). In Aptian–extant trionychids, the inner zone of the external cortex contains sheet-like structures piling up towards the external bone surface. In every sheet, assemblages of isotropically horizontal and vertical fibers (fiber bundle quadrangles or FBQs; Scheyer et al., 2007) are alternately accumulated horizontally, with more horizontal fibers than vertical fibers overall. The horizontal FBQs in every other sheet are arranged parallel to each other, forming a complex plywood-like organization (Fig. 4C, D). In Aptian–extant trionychid shell bones, this plywood-like structure consists of 7–18 sheets (Appendix 1), and the thickness of each sheet decreases towards the external bone surface. The contact between plywood-like structure and surficial parallel-fibered bone layer is discontinuous, being formed as a resorption line. Part of the trionychid shell bone material also possesses interwoven structural fiber (ISF) bundles, which are fiber bundles assembled without a plywood-like arrangement, in the transitional zone between the inner zone of the external cortex and the interior trabecular bone (Fig. 4F; Appendix 1). In these specimens, the fiber bundles gradually lose their plywood-like organization pattern internally. In other taxa of Trionychidae, this transitional zone is not observed because the base of the plywood-like structure is remodeled into secondary bone tissue. In Carettochelyidae, parallel-fibered bone tissue in the outer zone of the external cortex is internally succeeded by fine ISF or secondarily remodeled bone, not by a plywood-like layered structure (Fig. 4G–I).

Three costal specimens (FPDM-V0127, V1836, and V2761) from the Kitadani Formation exhibit a diploë structure, an external cortex with a peripheral parallel-fibred bone tissue layer, which may be accompanied by vertically inserted Sharpey's fibers and inner plywood-like microstructures (Fig. 5A, B, E; Appendix 1). The incomplete cross-section of FPDM-V0127 exhibits a thickened external cortex in the costal (Fig. 5E), and its compactness is 0.892. This value is relatively high compared with known costal compactness of turtles (0.395 in *Eileanchelys* and 0.563–0.603 in *Heckerochelys* [Scheyer et al., 2014]; 0.632–0.68 in *Yaminuechelys* [Janello et al., 2016]; 0.74–0.79 in *Dortoka* [Pérez-García et al., 2012]; 0.864–0.953 in *Solemys* [Scheyer et al., 2015]) but not exceptionally high or low among trionychian costals (0.906 in an Aptian–Albian trionychid [Fig. 5F]; 0.912 in a Cenomanian trionychid [Fig. 5G]; 0.825 in extant *Pelodiscus sinensis* [Fig. 5H]; and 0.912 in extant *C. insculpta* [Fig. 5I]).

The plywood-like microstructure consists of 7–18 horizontal sheets, and the layer thickness is reduced towards the external surface, as also observed in other Aptian–extant trionychids (Appendix 1). In total, the variations in general shell bone microanatomy, general histology, and the number and maximum thickness of bony sheets in the plywood-like microstructure in the FPDM trionychians fall within the histological variation observed in the Aptian–extant trionychids described above. A costal specimen of *K. okurai* from the Akaiwa Formation (SBEI 1728, Hauterivian–Aptian) possesses a diploë structure; however, the boundary between the compact cortex and the inner cancellous bone is indistinct. The external cortex consists of primary parallel-fibred bone but lacks the plywood-like organization of fiber bundles. The cancellous bone is almost completely occupied by secondary osteons. The primary bone tissue in the cancellous bone shows random orientation of its

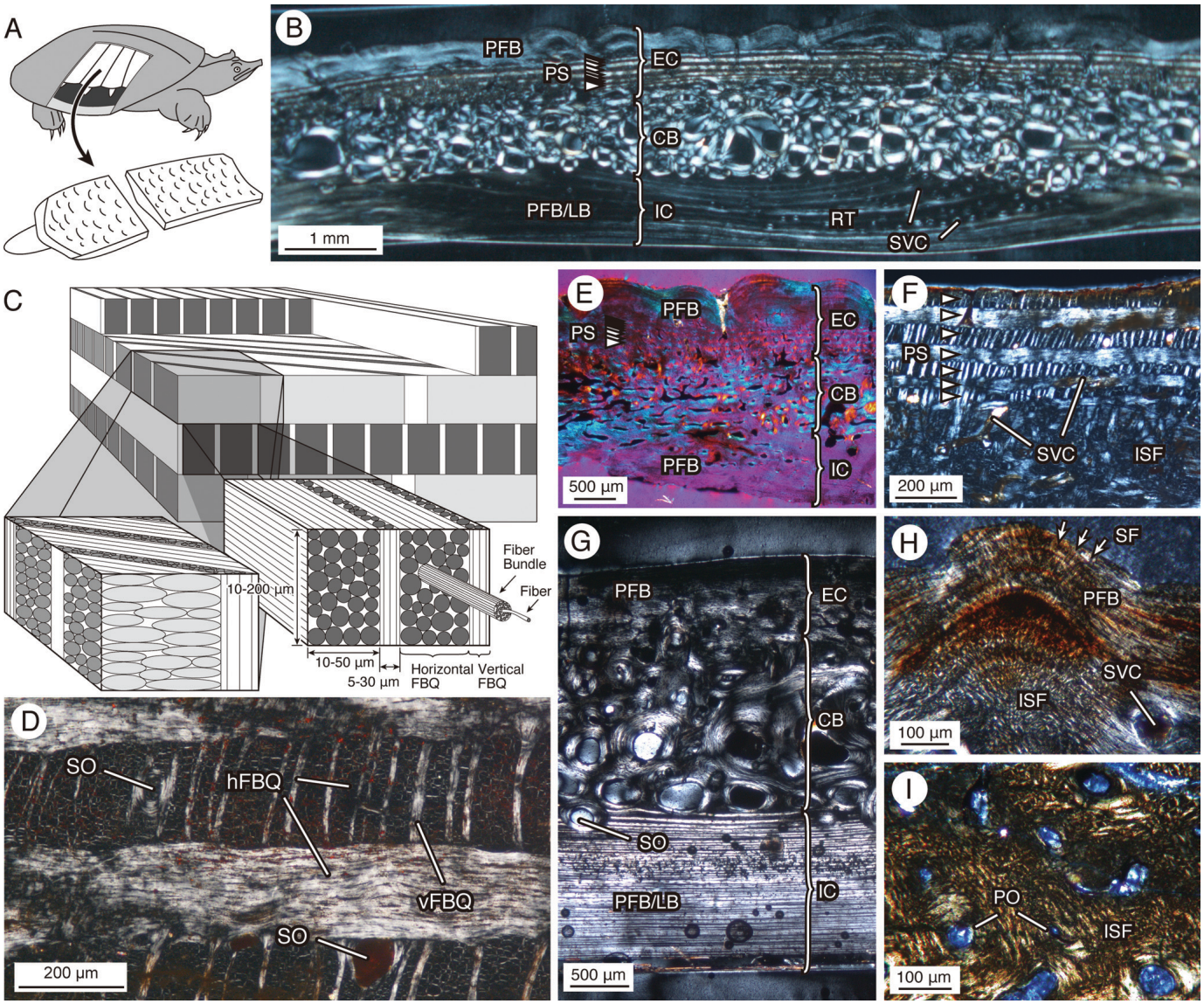

FIGURE 4. Histology of Aptian–extant trionychian shell bones. **A**, sampling location from a costal; **B**, extant trionychid *Apalone spinifera*, cross-sectional overview of the left fifth costal; **C**, schematic illustration of the general plywood-like microstructure in shell bones of trionychids, modified following Scheyer et al. (2007); **D**, a close-up view of the plywood-like microstructure in '*Trionyx*' *kansaiensis* (Trionychidae) from the Santonian of Kazakhstan, ZIN PH 751/64, hypoplastron; **E**, **F**, '*Trionyx*' *kyrgyzensis* (Trionychidae) from the Aptian of Kyrgyzstan, cross-sectional overview of an indeterminate shell fragment (ZIN PH 2/182) (**E**) and an external cortex of a costal (ZIN PH 3/182) (**F**); **G**, the extant carettochelyid *Carettochelys insculpta*, right seventh costal; **H**, *Anosteira pulchra* (Carettochelyidae) from the Eocene of Wyoming, UCMP V78031/131731, peripheral, external cortex, with arrows indicating the orientation of Sharpey's fiber insertion; **I**, *Allaeochelys* cf. *A. crassesculpta* (Carettochelyidae) from the Eocene of Germany, HLMD-Me10468, costal, close-up view of inner zone of the external cortex. Each triangle represents a single layer in the plywood-like microstructure. **Abbreviations**: **CB**, cancellous bone; **EC**, external cortex; **h(v)FBQ**, horizontal (vertical) fiber bundle quadrangles; **IC**, internal cortex; **ISF**, interwoven structural fiber; **LB**, lamellar bone tissue; **PFB**, parallel-fibered bone tissue; **PO**, primary osteon; **PS**, plywood-like structure; **RT**, rib thickening; **SVC**, simple vascular canal; **SF**, Sharpey's fiber; **SO**, secondary osteon. All thin sections were photographed in polarized light, except **E**, which was imaged in cross-polarized light using a lambda compensator. White arrowheads in **B**, **E**, and **F** indicate the number of plies within the PS.

fiber bundles, and the internal cortex shows a lamellar structure, as in carettochelyids (Fig. 5C, D).

DISCUSSION

The Trionychid Affinity of the Hauterivian–Aptian Specimens from Japan

The results of our histological analysis, in addition to the known shell bone microstructure of other turtles (e.g., Scheyer, 2007; Scheyer and Sander, 2007; Scheyer et al., 2007, 2014; Vlachos et al., 2015; de Buffrénil et al., 2016), allows us to confirm that the presence of a plywood-like bone microstructure is diagnostic only of Trionychidae (Fig. 6). This shell bone microstructure is acquired through metaplastic ossification of similarly organized collagen fiber bundles in the dermis, which takes place on the edge of shell bones and likely reinforces the shell plate (Scheyer et al., 2007). On the basis of current phylogenetic hypotheses, the plywood-like bone microstructure is seemingly acquired after reduction of keratinous scales as an alternative reinforcing tissue of shell (Fig. 6). Other synapomorphies in the

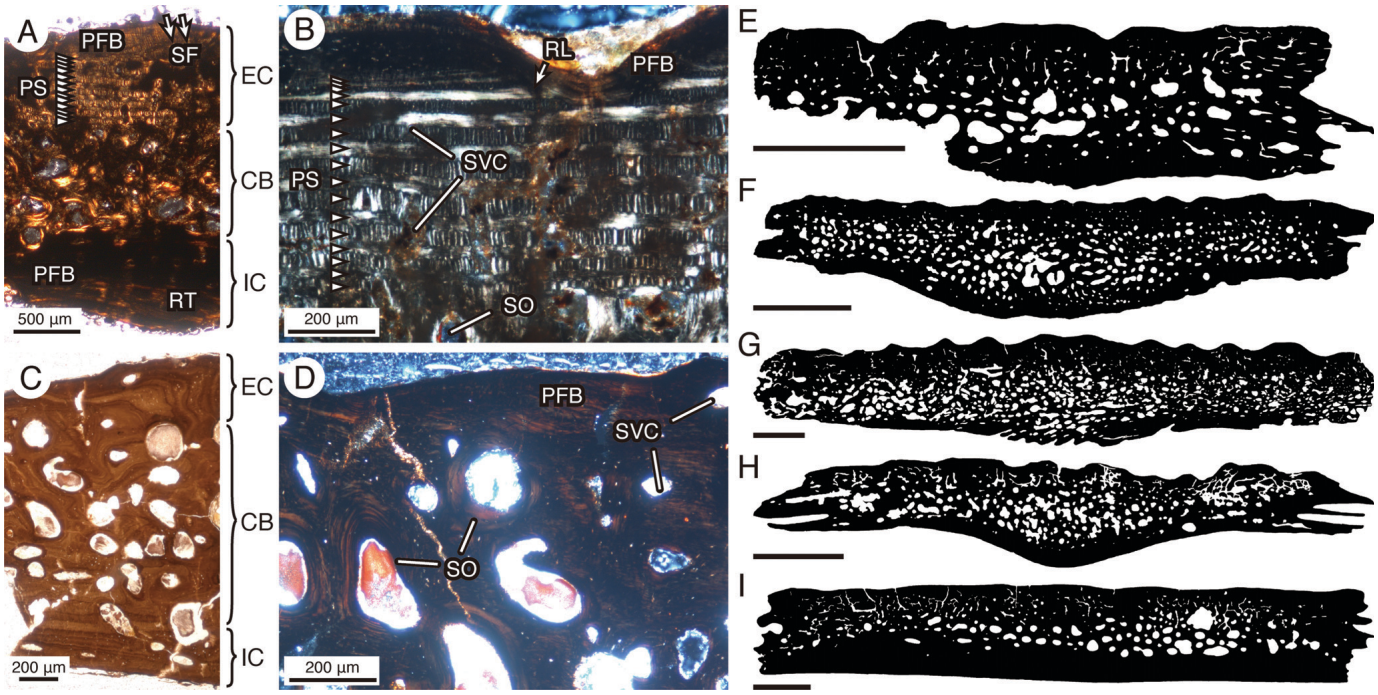

FIGURE 5. The costal bone histology and microanatomy of Hauterivian–Aptian trionychians from the Tetori Group, Japan, with comparative microanatomical images from trionychian costal cross-sections. **A**, **B**, specimens from the Barremian–Aptian Kitadani Formation, partial cross-section of FPDM-V1836 (**A**) and close-up image of the external cortex in FPDM-V0127 (**B**); **C**, **D**, *Kappachelys okurai* right seventh costal, SBEI 1728, Hauterivian–Aptian Akaiwa Formation, partial cross-section (**C**) and a close-up image of the external part (**D**). Thin sections were photographed in cross-polarized light (**A**, **B**, and **D**) or in natural light (**C**). **E**–**I**, binarized images of trionychian costal cross-sections. **E**, FPDM-V0127 (position unknown); **F**, ZIN PH 102, Trionychidae, gen. et sp. indet., Aptian–Albian of Mongolia (position unknown); **G**, ZIN PH 122, Trionychidae, gen. et sp. indet., early Cenomanian of Uzbekistan (position unknown); **H**, *Pelodiscus sinensis* (Trionychidae, extant), left fourth; **I**, *Carettochelys insculpta* (Carettochelyidae, extant), right seventh. **Abbreviations**: **CB**, cancellous bone; **EC**, external cortex; **IC**, internal cortex; **ISF**, interwoven structural fiber; **LB**, lamellar bone tissue; **PFB**, parallel-fibered bone tissue; **PS**, plywood-like structure; **RL**, resorption line; **RT**, rib thickening; **SF**, Sharpey's fibers; **SO**, secondary osteon. Note that the white arrow heads in **A** and **B** indicate the number of plies within the PS, whereas the white arrows in **A** indicate the insertion of SF. Scale bars equal 2 mm (**E**–**I**).

shell of Trionychidae (i.e., the complete absence of keratinous scutes; the reduction or complete loss of peripheral bones, pygal, or suprapygal; and the boomerang-shaped entoplastron (Joyce and Lyson, 2010) are difficult to recognize in isolated fragmentary bones, whereas the plywood-like microstructure was observed in all the shell bone elements of trionychids that we examined, except for *Kappachelys*. This highly organized bone microstructure is not likely to appear convergently with high frequency; therefore, its presence should be a reliable criterion to identify fragmentary trionychid shell material. We also found a transitional bone tissue between ISF and the plywood-like structure accompanied by the typical plywood-like structure from fossil trionychids; however, this does not seem to indicate the plesiomorphic condition because it is found not only in (potential) trionychids with many other plesiomorphic attributes, but also in trionychids that exhibit many derived traits, such as *Lissemys*. This transitional tissue seems to be possessed by all trionychids deep to the typical plywood-like structure; however, in most specimens the inner part is intensely remodeled and the primary microstructure is not preserved. The presence or absence of surficial parallel-fibered bone tissue is also variable among trionychids and is not indicative of their taxonomic status (Appendix 1). *Kappachelys* from the Hauterivian–Aptian of Japan does not exhibit a plywood-like microstructure; its shell microstructure is more similar to that of carettochelyids, a group that retains the plesiomorphic histology. The shell bone histology of *Kappachelys* suggests that this genus is the earliest-branching taxon among known trionychids, as Hirayama et al. (2012) discussed based on a number of plesiomorphic states, or that it is simply a carettochelyid (Fig. 6).

The de-scalation of the body surface is thought to improve the cutaneous respiration of turtles in resting conditions and to enable them to remain submerged for a longer time than scale-bearing turtles (Bagatto and Henry, 1999; Scheyer et al., 2007). The flat and reduced shell bones recognized in FPDM specimens are further advantageous for camouflage in the bottom sediment of fluvial or lacustrine environment, helping them to ambush prey or to avoid attacks by predators (Scheyer et al., 2007). It is also noteworthy that trionychids, including the Barremian–Aptian taxon (taxa) from Japan, contrast in their shell morphology with highly terrestrial turtles (e.g., Testudinidae and Nanhsiungchelyidae) in which robust shell bones also exhibit deep scute sulci (Fig. 6; Hirayama et al., 2001; Brinkman, 2005).

The high shell bone compactness in turtles is generally considered as evidence of a terrestrial mode of life (Scheyer and Sander, 2007; Scheyer et al., 2014, 2015). However, costal bones of extant trionychians, a group that is highly adapted to non-marine aquatic habitats, exhibit high compactness that is clearly not a result of terrestrial adaptation. Increase in bone mass (pachyostosis sensu lato) is commonly found in tetrapods that dwell in shallow-water environments (Houssaye, 2009; Scheyer et al., 2014). A pachyostotic skeleton would act as ballast and allow passive (hydrostatic) control of the body under water, rather than active (hydrodynamic) control for swimming (de Ricqlès and de Buffrénil, 2001). Interestingly, limb bones are less compact in trionychids compared with those of semiaquatic

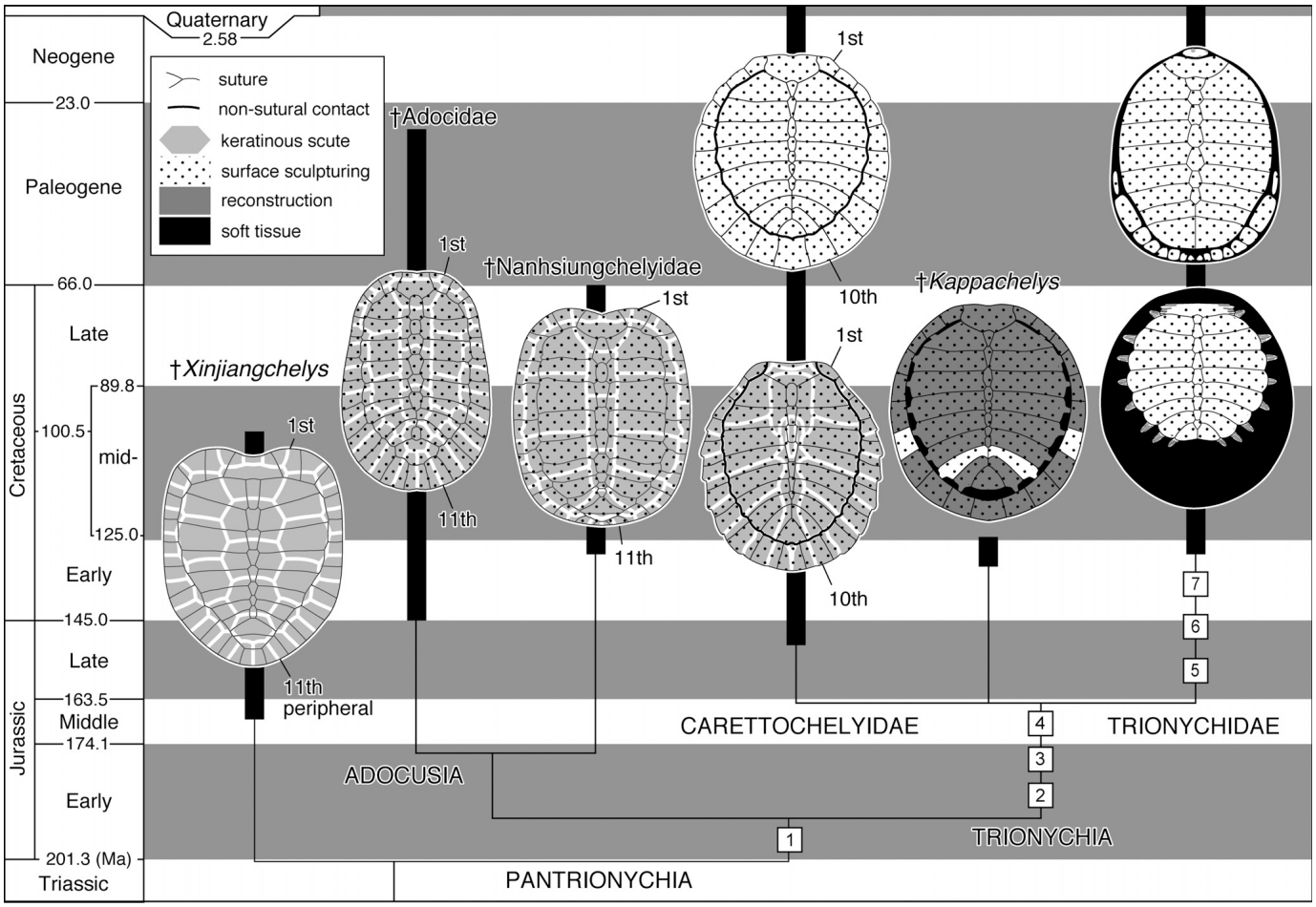

FIGURE 6. Evolution of the carapacial structure of Pantrionychia. The seven characteristics (1, a shell sculpture; 2, 20 or fewer peripheral bones; 3, a partial loss of keratinous scutes in carapace; 4, a loss of the costal-peripheral suture; 5, a complete loss of the keratinous scutes; 6, a reduction or loss of the peripherals; and 7, plywood-like shell microstructure) are mapped onto a time-calibrated cladogram that was consistently compiled following Meylan (1988), Joyce (2007), and Hirayama et al. (2012). Skeletal reconstruction of *Kappachelys* is based on known specimens and *Carettochelys insculpta*. Keratinous scutes on the carapace are reduced in carettochelyids (e.g., *Kyzylkumemys*; below) and disappear in adult *C. insculpta* (above). In Trionychidae, ossicles along the posterior shell margin may be present (*Lissemys*; above) or absent (other trionychids, e.g., *Trionyx*; below). *Sinaspideretes* (i.e., *Yehguia*) and *Peltochelys* are not included in this tree because of their controversial systematic positions.

and terrestrial turtles (Nakajima et al., 2014), showing a different trend to this study, which is based on shell bones. Considering that shell bones apparently occupy the largest proportion of skeletal mass in a turtle, we can conclude that the high skeletal mass of trionychids, including Barremian–Aptian taxa from Japan, likely represents an adaptation to a benthic lifestyle and ambush predation. In conclusion, the bone morphology and histology of the new specimens from the Barremian–Aptian in Japan confirm their trionychid affinity, and simultaneously, they also suggest a high degree of aquatic adaptation.

Paleobiogeography

The FPDM specimens constitute one of the oldest records of Trionychidae during the Barremian–Aptian interval. In addition to this and *Kappachelys* from the Hauterivian–Aptian of Japan (Hirayama et al., 2012), possible stem trionychids have been reported from China (Li et al., 2015a, 2015b); however, no trionychids are yet known from other regions prior to the Albian (Fig. 7). This is not direct evidence of an eastern Asian origin of Trionychidae because the fossil record of non-marine turtles from the Hauterivian–Barremian is generally quite incomplete; however, an Asian origin is supported by the lack of trionychids in Lower Cretaceous non-marine turtle fossil localities outside Central and East Asia. Trionychids have also been reported from unnamed formations in Alxa, Inner Mongolia (possibly Lower Cretaceous), and Shine Usny Tolgod and Dzun Shakai of eastern Mongolia (possibly Lower Cretaceous), even though these occurrences may be younger than Aptian (Danilov and Vitek, 2012; Danilov et al., 2014). Albian trionychids are found in the area now occupied by the Fergana Depression in Central Asia. In North America, trionychids first appear in the Cenomanian (Brinkman, 2003). 'Trionychidae indet.' has been reported from the Mussentutit Member of the Cedar Mountain Formation in western Utah, a unit that is often referred to as Albian in age (Fiorillo, 1999), but its absolute age (96.7–98.5 Ma; Garrison et al., 2007) corresponds to the Cenomanian in the latest International Chronostratigraphic Chart (Cohen et al., 2013). In the Cenomanian–Turonian interval, trionychids were distributed widely in the Northern Hemisphere except in Europe, where the oldest record of trionychids is from the latest early Campanian (approximately 80 Ma) (Scheyer et al., 2012).

On the basis of the Asian origin hypothesis of trionychids, they likely spread progressively into central Eurasia and North America during the Cenomanian–Turonian (Fig. 7A–C). The mid-latitude average temperature was lower than that of the present

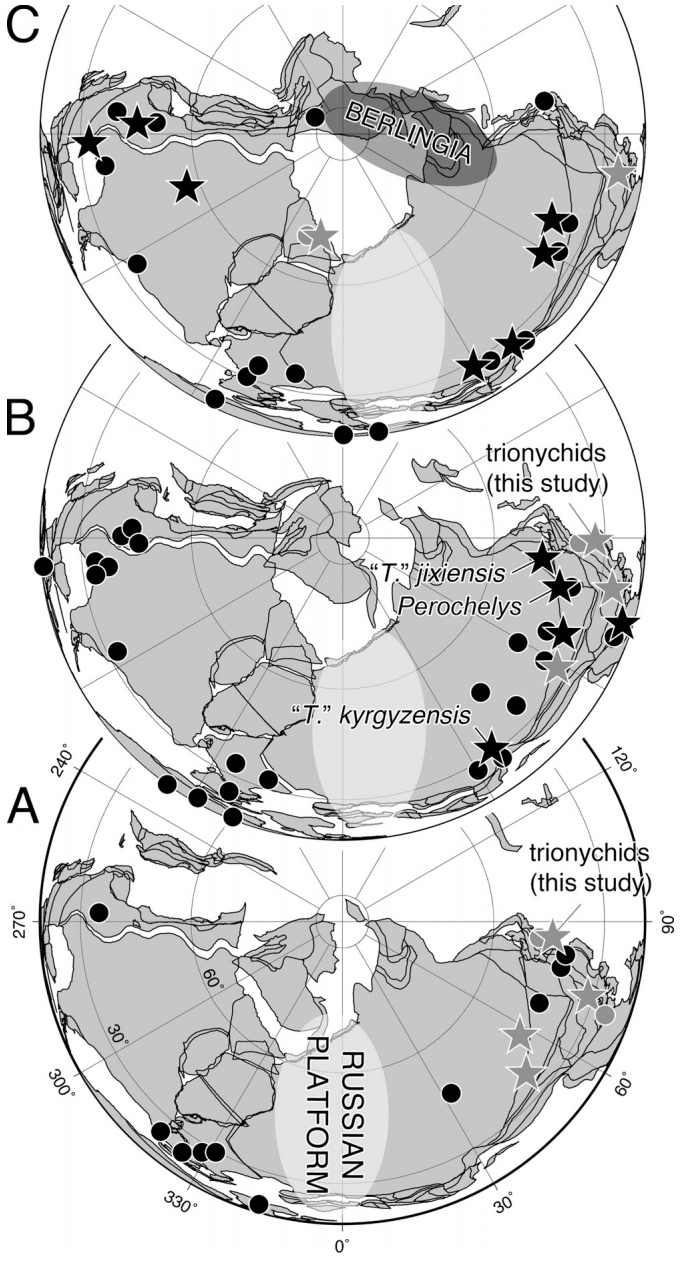

FIGURE 7. The paleobiogeographic distribution of non-marine turtles during the late Early to early Late Cretaceous (Hauterivian–Turonian), with special reference to trionychids. Fossil localities are plotted on 130 (**A**), 110 (**B**), and 90 (**C**) Ma (north) polar-orthographic paleomaps, representing the Hauterivian–Barremian, Aptian–Albian, and Cenomanian–Turonian occurrences, respectively. Stars denote trionychids, whereas circles denote non-trionychid and indeterminate freshwater turtles. Black marks indicate localities within the age range of the corresponding map, whereas the gray marks indicate localities where the estimated age range exceeds the earliest or the latest limit of the corresponding map.

day during the Barremian; however, as global CO_2 levels increased in the Early Cretaceous, the mid-latitude average temperature began to increase locally in eastern Asia from the Aptian (Pucéat et al., 2003; Steuber et al., 2005) or possibly from the late Albian onwards (Amiot et al., 2011). As a result of global warming, the humid belt spanned 30–50°N or a wider area northwards during the Aptian–Turonian at least in eastern Asia (Hasegawa et al., 2012), an area that includes the majority of trionychid fossil localities (Fig. 7B, C). Because FPDM trionychids might not have scales or keratinous scutes to protect their skin from evaporation, permanent water is largely necessary for their survival, as is the case with extant trionychids. Therefore, the expansion of fluviolacustrine environments helped trionychids disperse widely. Trionychids have a Cenomanian fossil record in western North America and Asia, but they were absent in Europe, suggesting that eastward migration of Asian trionychids into North America took place via Beringia (Hirayama et al., 2000; Hutchison, 2000) during the Albian–Cenomanian interval when the polar ice was progressively receding (Fig. 7C; Pucéat et al., 2003; Hay and Floegel, 2012). Conversely, the fossil record does not support the westward migration of trionychids from Laurasia into North America. This may simply be due to the lack of a non-marine turtle fossil record from the Cretaceous of eastern Europe, or to the intermittent presence of seaways that connected the Arctic and Tethys oceans through the Russian Platform in accordance with sea level fluctuations during the Cretaceous (Fig. 7; Baraboshkin et al., 2003; Košťák and Wiese, 2008).

These geological and geographical records imply that the mid-Cretaceous greenhouse climatic conditions may have contributed to the widespread radiation of Trionychidae, which has lasted until the present. It is important to compile fossil records and paleoclimatologic data sets, especially with increasing spatiotemporal resolution in the future, to increase our understanding of the origin of the present-day freshwater turtle fauna.

CONCLUSIONS

From osteological and osteohistological analyses of the oldest trionychid candidates from the Kitadani Formation of Japan, it was confirmed that the Trionychidae was present in the Barremian–Aptian on the eastern margin of Asia in what is now southeastern Japan, and that these earliest trionychids already had morphological features showing trionychid affinities. The shell bone tissue of the Kitadani specimens shows a characteristic plywood-like microstructure and falls within the histological variation of Aptian–extant trionychids. *Kappachelys* from the Hauterivian–Aptian Akaiwa Formation of Japan, conversely, does not show plywood-like bone microstructure in its costal bone, suggesting that this taxon could be the earliest-branching stem trionychid or a carettochelyid. A high degree of aquatic adaptation and a benthic lifestyle are also suggested from the skeletal morphology of the Early Cretaceous trionychids (a flat, flexible, and de-scaled shell).

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APPENDIX 1. Histological samples of trionychian turtle shells examined in this study. **Abbreviations**: **PFB**, presence of external parallel-fibered bone; **PS**, number of plywood-like layers; **PS/ISF**, presence of plywood-like structure/interwoven structural fiber transitional tissue; +, present; −, absent; **?**, difficult to determine because of lack of information or poor state of preservation. *See Institutional Abbreviations.

Taxon	Locality and remarks	Inventory no.*	Sectioned elements	PS	PS/ ISF	PFB
Carettochelyidae						
Carrettochelys insculpta	Captive (extant)	WU-SILS-RH1044	Right 7th costal	−	−	+
	Locality unknown (extant)	MAGNT R12640	Peripheral	−	−	?
			Neural	−	−	+
			Hypoplastron	−	−	?
Allaeochelys cf. *crassesculpta*	Messel pit, Germany (Middle Eocene)	HLMD-Me10468	Costal	−	−	?
			Peripheral	−	−	?
Anosteira pulchra	Washakie Fm., Wyoming (Eocene)	UCMP V78031/ 131731	Costal	−	−	?+
			Peripheral	−	−	+
			Plastral fragment	−	−	?
Anosteira sp.	Uinta Fm., Wyoming (Late Eocene)	FM PR 819	Costal	−	−	+
Trionychidae						
Pelodiscus sinensis	Okinawa, Japan (extant)	NSMT-H 6600	Costal	8	−	+
Apalone spinifera	Unknown (extant)	YPM 13874	Left 5th costal	11	−	+
Aspidoretoides riabinini	Yalovach Fm., Tajikistan (early Santonian)	ZIN PH 922/64	Costal	14	?+	+
		ZIN PH 639/64	Nuchal	?9	?	?−
		ZIN PH 710/64	Hypoplastron	9	?	+
'*Trionyx*' cf. *kansaiensis*	Bissekty Fm., Uzbekistan (late Turonian)	collection ZIN PH 108	8th costal	16	−	+
Aspidoretoides cf. *riabinini*		ZIN PH 382/108	8th costal	15	−	+
		ZIN PH 428/108	Nuchal	?9	?+	+
'*Trionyx*' *kansaiensis*	Yalovach Fm., Tajikistan (early Santonian)	ZIN PH 751/64	?Hypoplastron	12	?+	+
		ZIN PH 893/64	Costal	10	?	+
'*Trionyx*' *kyrgyzensis*	Upper Almyshik Fm., Kyrgyzstan (lower–middle Albian)	ZIN PH 1/182	Costal	12	+	+
		ZIN PH 2/182	Shell fragment	13	+	+ (thin)
		ZIN PH 3/182	Costal	15	+	−
		ZIN PH 4/182	Costal	15	?	+
		ZIN PH 5/182	Shell fragment	13	?	+
Lissemys punctata	Locality unknown (extant)	SMNS 3705	?Neural 3	?7	−	+ (thin)
	Locality unknown (extant)	YPM 11645	Marginal ossicle	13	+	?+ (thin)
			Plastral fragment	15	+	−
Helopanoplia sp.	Hell Creek Fm., Montana (?uppermost Cretaceous)	UCMP V87051/ 150193	Costal	8	+	+
Plastomenus sp.	Bridger Fm., Wyoming (Eocene)	UCMP V81110/ 150231	Costal	15	+	?−
gen. et sp. indet.	Kitadani Fm., Japan (Barremian–Aptian)	FPDM-V0127	Costal	?7	−	+
		FPDM-V1836	Costal	18	+	+
		FPDM-V2761	Costal	10	?+	+ (thin)
gen. et sp. indet.	Erdeni Ula locality, Mongolia (Aptian–Albian)	Collection ZIN PH 102	Costal	7	?+	+ (thin)
gen. et sp. indet.	Khodzhakul Fm., Uzbekistan (early Cenomanian)	Collection ZIN PH 122	Costal	13	−	+
			Costal	17	+	+
gen. et sp. indet.	Lower Baynshire Fm., Mongolia (Cenomanian–early Turonian)	Collection ZIN PH 80	Costal	11	−	+ (thin)
			Costal	?	?	?+
gen. et sp. indet.	Upper Baynshire Fm., Mongolia (late Turonian–Santonian)	Collection ZIN PH 167	Costal	10	?	+
gen. et sp. indet.	Nemegt Fm., Mongolia (Maastrichtian)	Collection ZIN PH 157	Costal	18	−	+
			Costal	12	?−	+
Family incertae sedis						
Kappachelys okurai	Akaiwa Fm., Japan (Hauterivian–Aptian)	SBEI 1728	Right 7th costal	−	−	+